

US EPA ARCHIVE DOCUMENT

TESTING REPELLENTS FOR INDUSTRY

A contract lab's methods



Repellent Testing

GLP vs. non-GLP

- In vitro & in vivo lab tests usually non-GLP:
exploratory data
- Field Tests GLP for EPA:
confirmatory data

In vitro

- 'Gupta box'
- Membrane-covered blood-filled wells
- Caged mosquitoes
- Preliminary screen
- No safety data needed

In-vitro Procedure

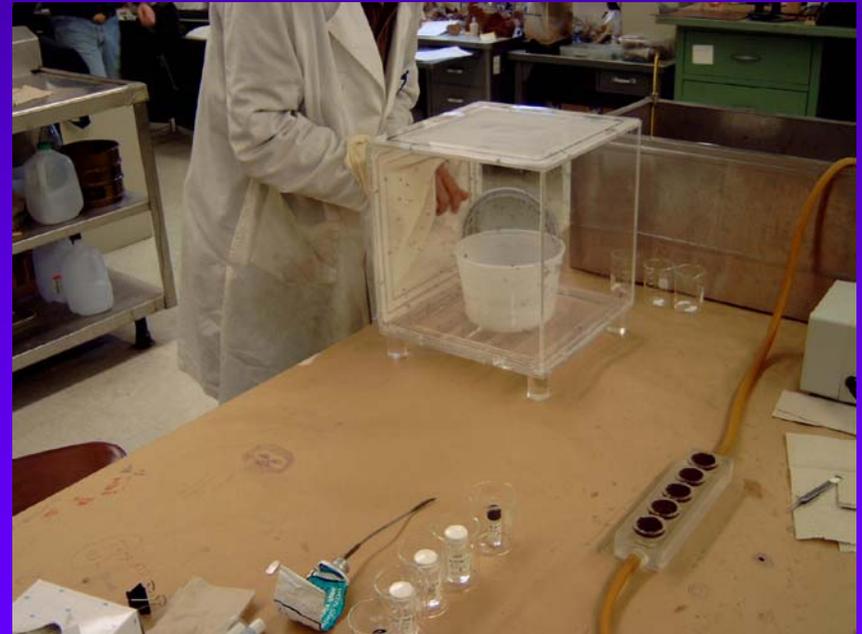
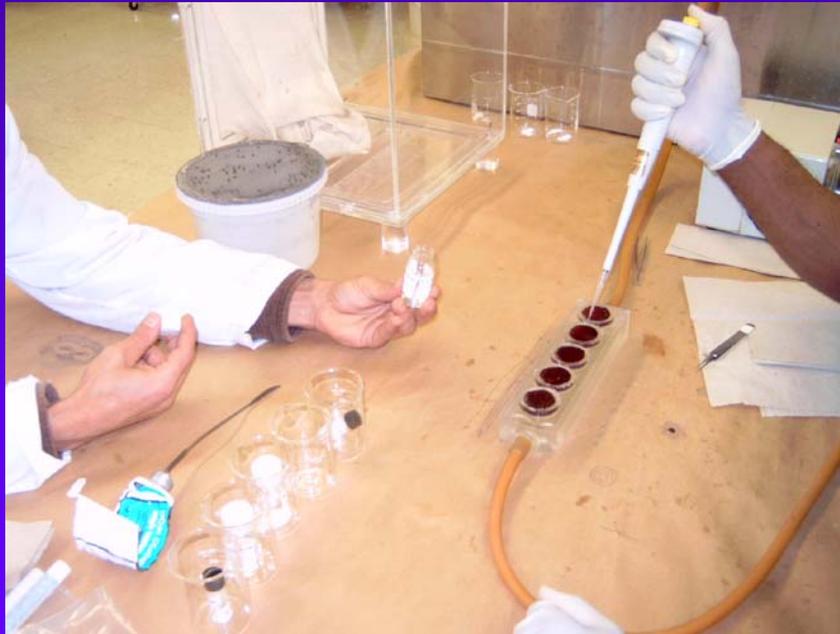
- Warm blood
- Fill wells
- Apply membrane,
silicone grease to
stick it down



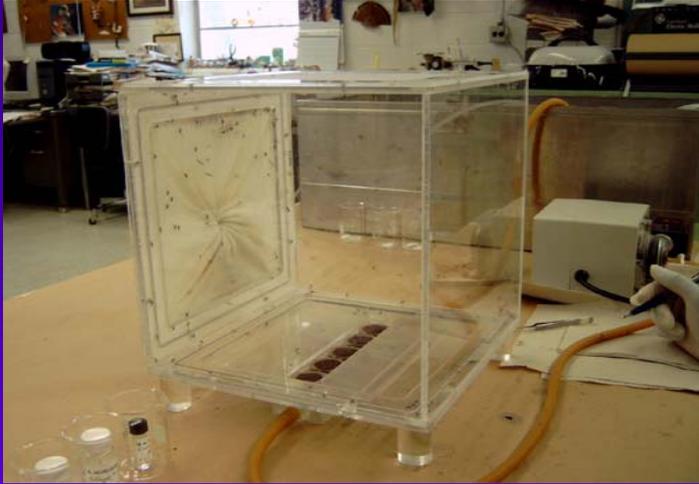
In-vitro Procedure (cont.)

Treat membranes
25 μ l/well

Release mosquitoes



In vitro Method (cont.)



- Begin test
- Count probes every 2 mins for 20 mins



In vitro Results

- Compares probe rates
 - % repellency
- Suitable for new AI's
- 3 experimental repellents or dilutions
- 1 standard repellent
- 1 control

In vivo

- Human test subjects
- Paid

In vivo

Human Test Subjects

- 21 CFR 45 & 46
- Institutional Review Board (IRB) Review
 - Essex IRB (Lebanon, NJ)
- Informed consent document
- Protocol
- Indemnification of ICR and IRB
- IRB
 - Protects subjects
 - fully informed
 - in layperson's English
 - Protects Sponsor
 - Protects Testing Company

But lots of extra paperwork and time!

In vivo-Laboratory Application



- 250 cm² area on forearms
- Measured – arms vary
- 1.67 mg/cm² (EPA)
- Range: 0.2 - 1.0 ml
- Pipetted
- Rubbed on

In vivo - Laboratory

- Modified USDA method
 - 2 x 2 x 2 ft cage
 - 4 sleeved entrances
 - 2 subjects per cage
 - 25 - > 1000 mosquitoes per cage
 - *Aedes aegypti*
 - *Culex quinquefasciatus*
 - *Anopheles* sp being obtained



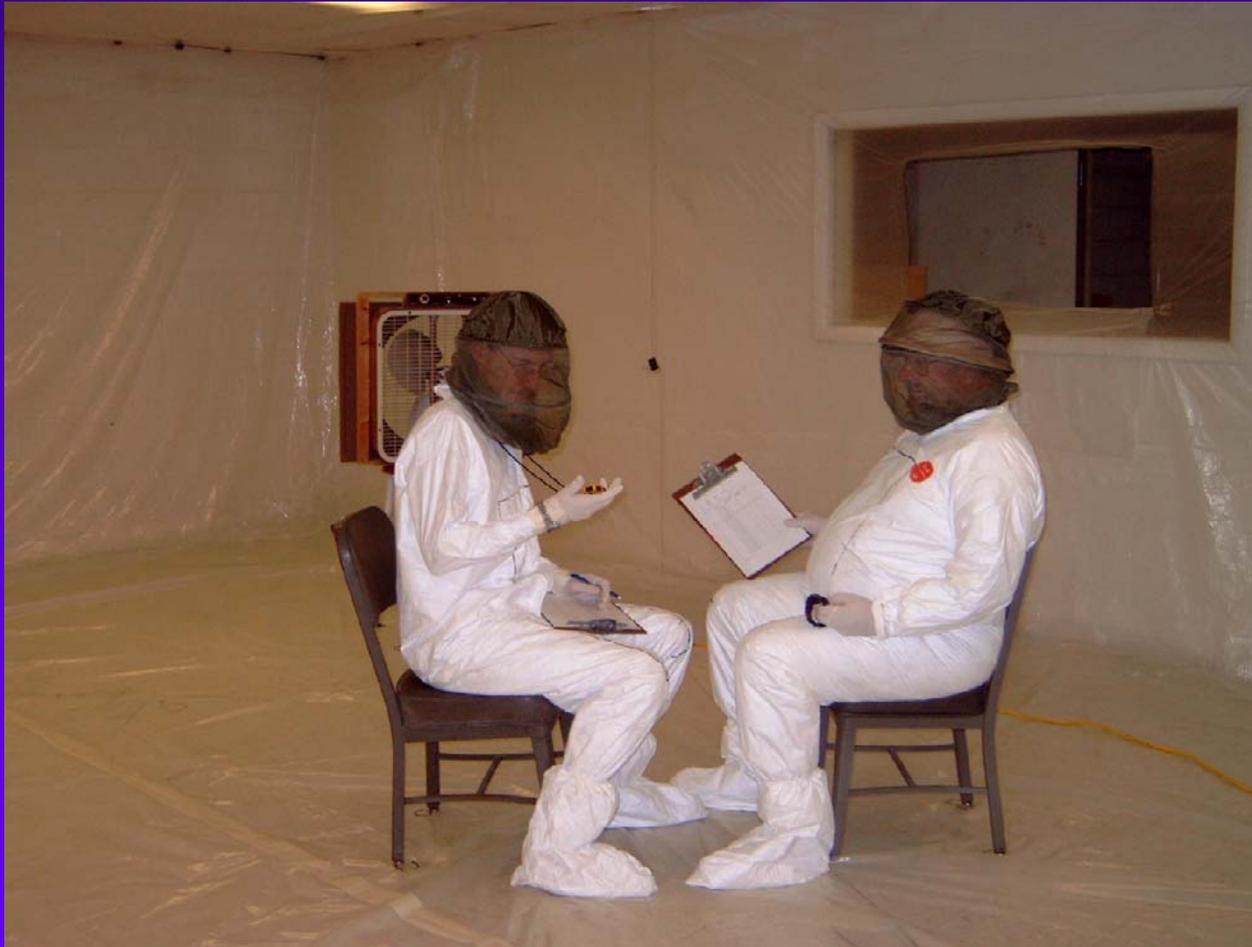
In vivo-Laboratory (cont.)

- Procedure
 - Up to 8 hours (or more) or breakdown
 - 5-minute exposures every 30 minutes
 - Breakdown = First Confirmed Bite
 - 1 bite followed by 2nd in 30 minutes
 - Bite = abdominal swelling
 - Probes
 - Probes + Landings for stable flies

In vivo - Laboratory Results

- longevity of complete protection
- 2 products per volunteer subject
- Controls:
 - Pre-test landing rates
 - Technician exposure – landings
- Individual variation

Repellent devices

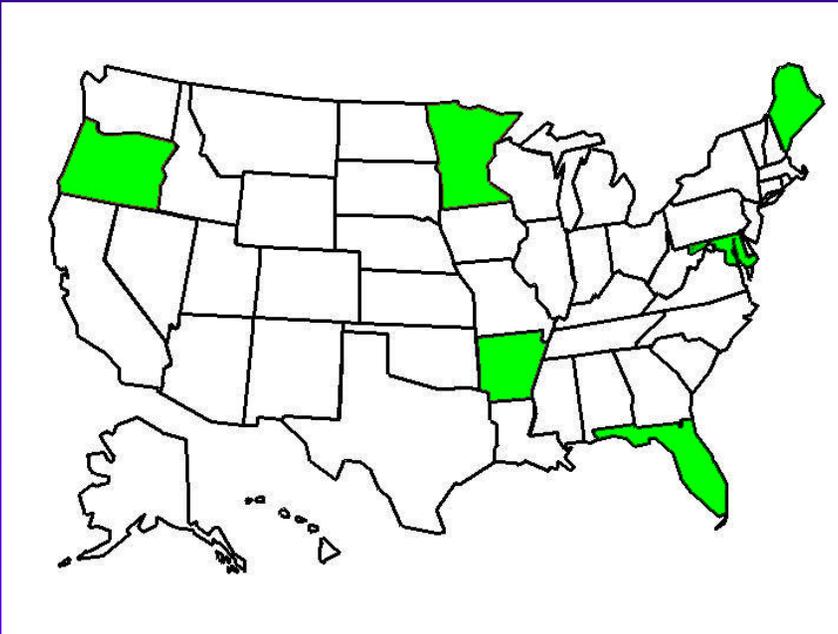


Field Trials

- Greater realism
- Greater cost
- More time to conduct
- EPA to require in new guidelines



Locations



- Florida - Everglades
- Maine – central
- Maryland Eastern Shore - Assateague
- Arkansas- Stuttgart
- Oregon – Portland
- Minnesota – s.east
- Washington - east
- Texas - Gulf Coast

Land Owner Etiquette

- Advance permission
- State and national parks, private land
- Permits – national parks
- Collecting – mosquitoes only
- Reference specimens to national parks
- Annual report – national parks
- Keep out of their way
- Say ‘Thank you’

subjects

- Local – recruit in advance
 - Reliability.
- Bring ICR Recruits – fly them out
 - Logistics
 - Cost
- IRB



Field Trial Logistics

- Locating field site
- Choosing dates
- Local guides
 - MADs
 - Outdoorsmen
 - Universities
- Recruitment of subjects (for ca. one week)
- Booking flights, vans
- Accommodation – motel, lodges
- Bandages, tape, coolers, ‘field food’
- Data forms, samples
- etc

Field Trial Chronology

- Travel day
- Scouting day
 - Location of mosquito activity
 - Activity peak period
- Testing days (1-4)
- Foul weather
- Travel Day

Field Trial (cont.)

- Application in the field (top loader + syringe)
- Volume easier than weight
- Organization/discipline 'Don't talk all at once'
- KISS - A, B, C etc



Test Days



- Early start
- Monitoring bites
- 2 Controls – land rates
- Whole body counts = activity confirmation
- If activity falls – move on
- If 0 activity - move out of state!
- Bad weather - tarps

Test Days (cont.)



Looking after subjects

- Keep them hydrated
- Keep the fed
- Keep them safe
- Keep them entertained
- Keep them in line ('Don't rub your arms!')



Field Data



- **Duration of protection – arms / legs**
 - time to first confirmed bite (2 in < 30 mins)
- **Continuous exposure to 4 – 8+ hours**
- **Controls – whole body landing rates to confirm mosquito activity**
 - Bites on arm / leg

Field Data (cont.)

- 2 controls
- 10 test subjects (varies)
- Study director and 2-4 ICR staff
- Q/A observes
- Weather conditions
- Mosquitoes sampled and identified after test – by a BCE

Final Report and EPA Inspections

- Protocol, raw data and all calculations
- GLP: draft to sponsor before finalized

■

THANK YOU